FIG. 1

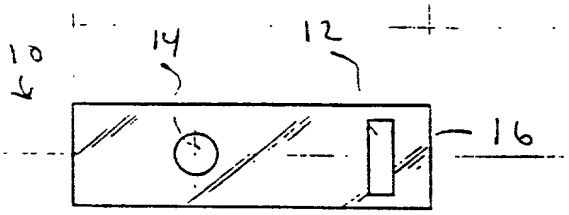


FIG. 2

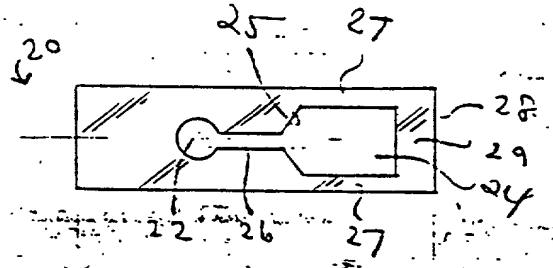


FIG 3

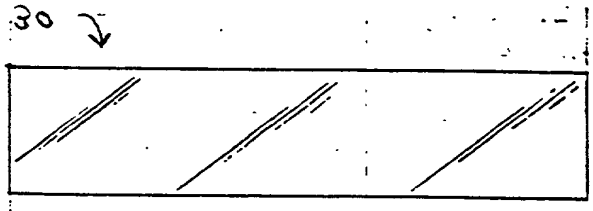
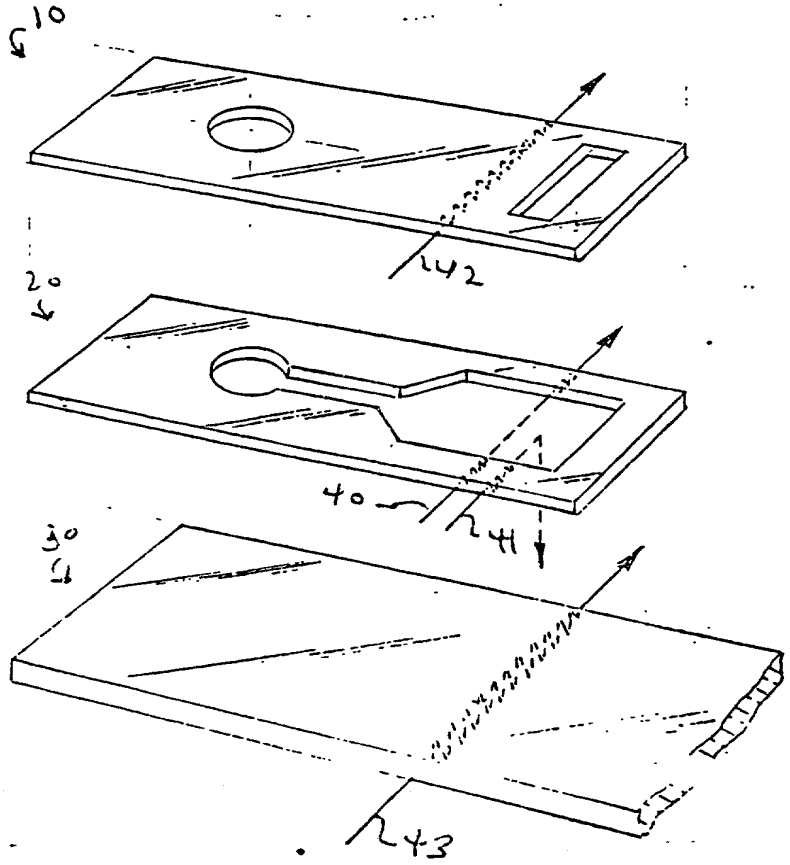
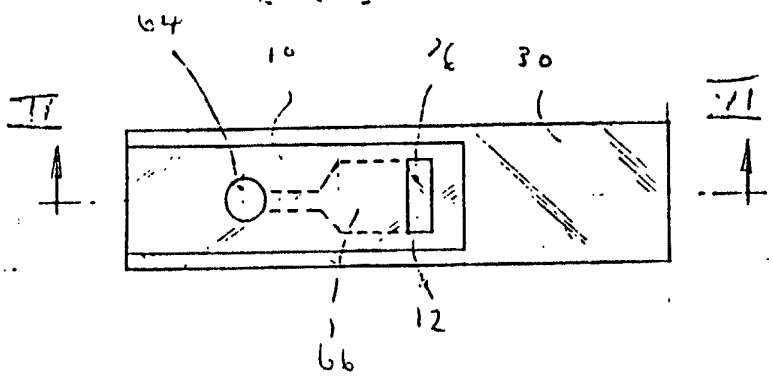


FIG. 4



033-CONT/4

FIG. 5



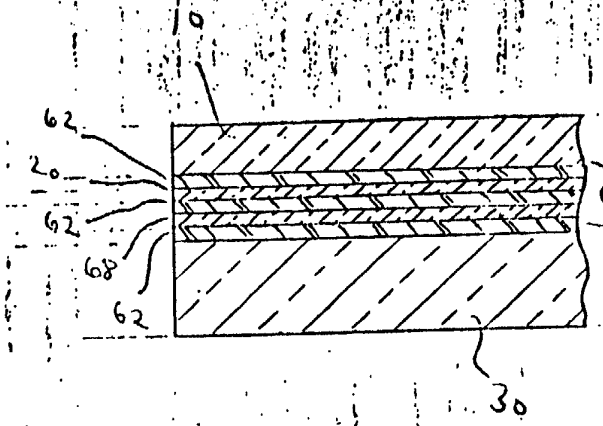


FIG. 7

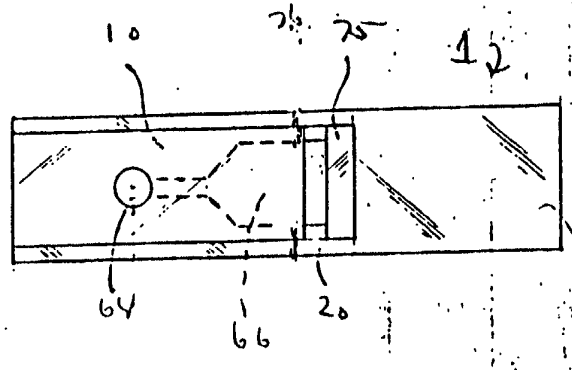


FIG. 8

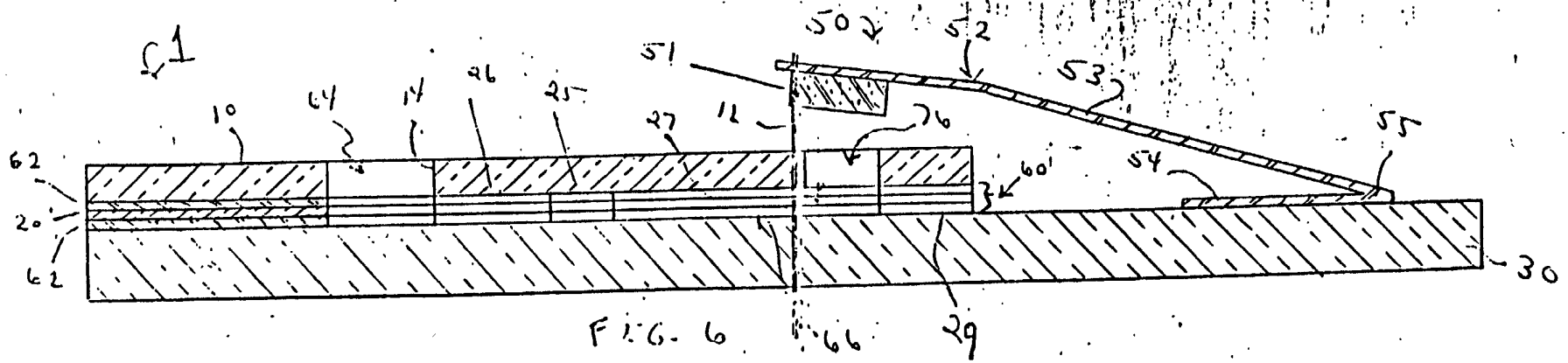


FIG. 6

FIG 9

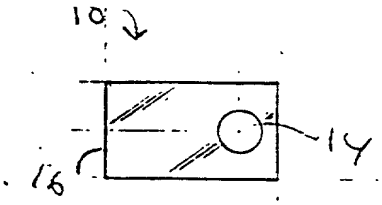


FIG 10

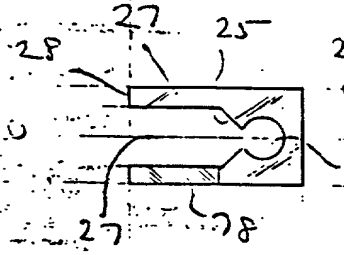


FIG 11

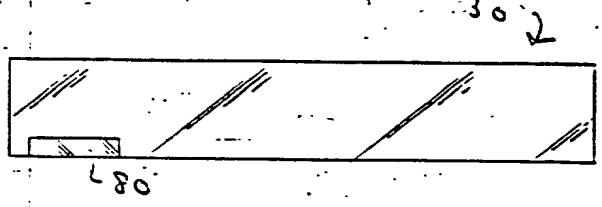


FIG 12

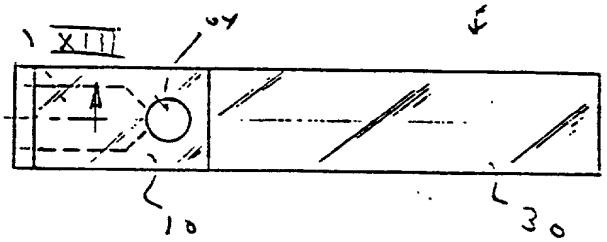
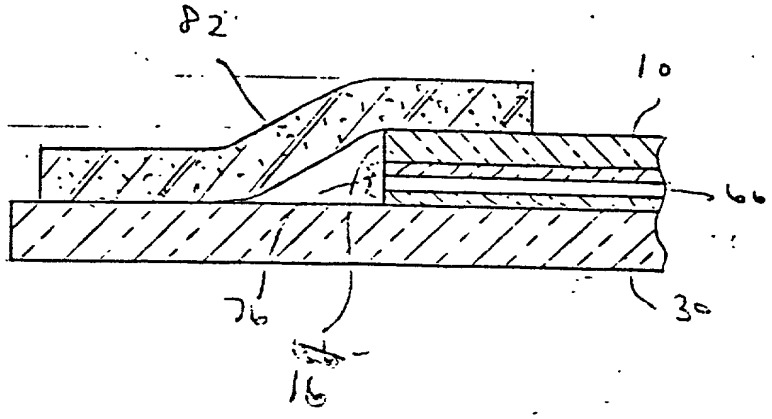


FIG 13



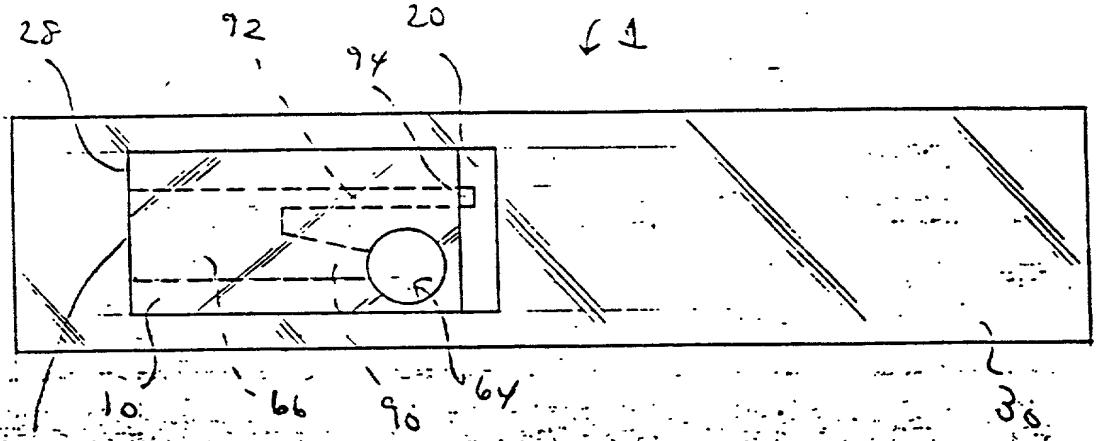

28
92
94
20
10
66
90
64
30
6

FIG 14

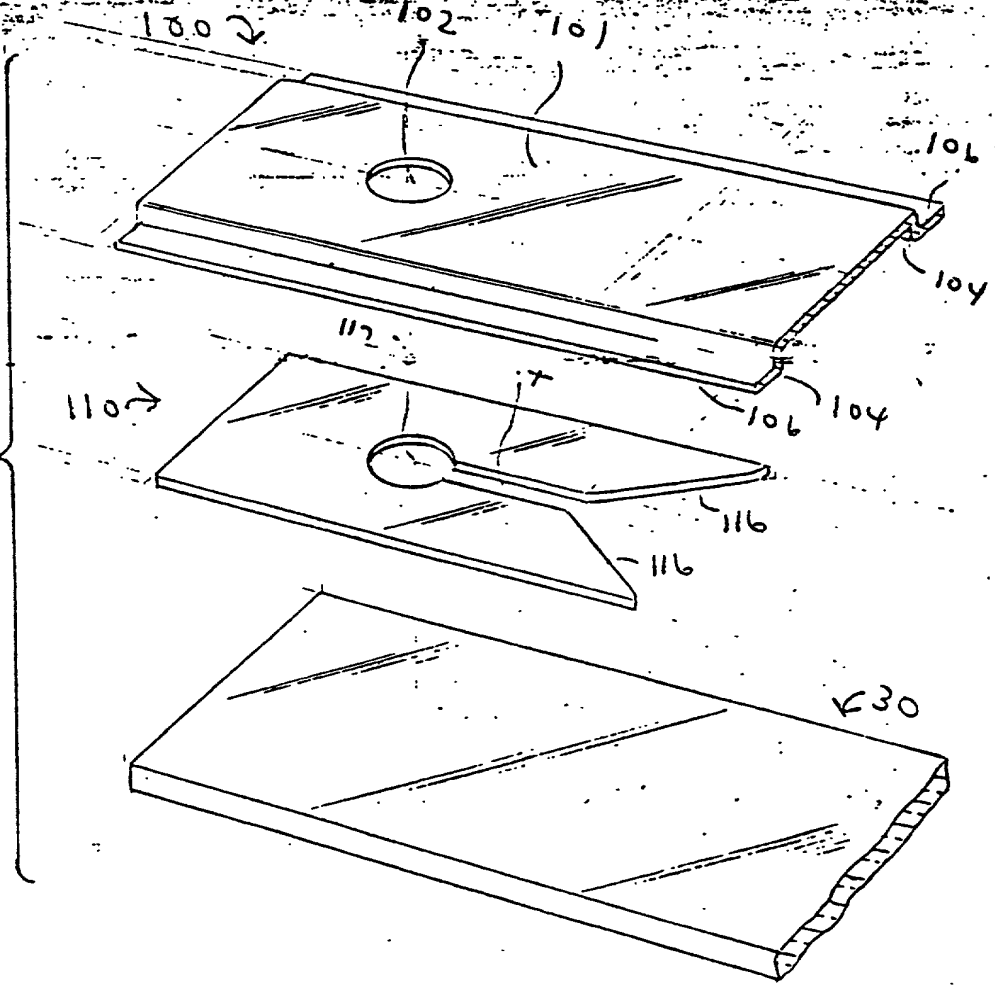

100
102
101
104
104
106
112
110
116
116
130

FIG 15

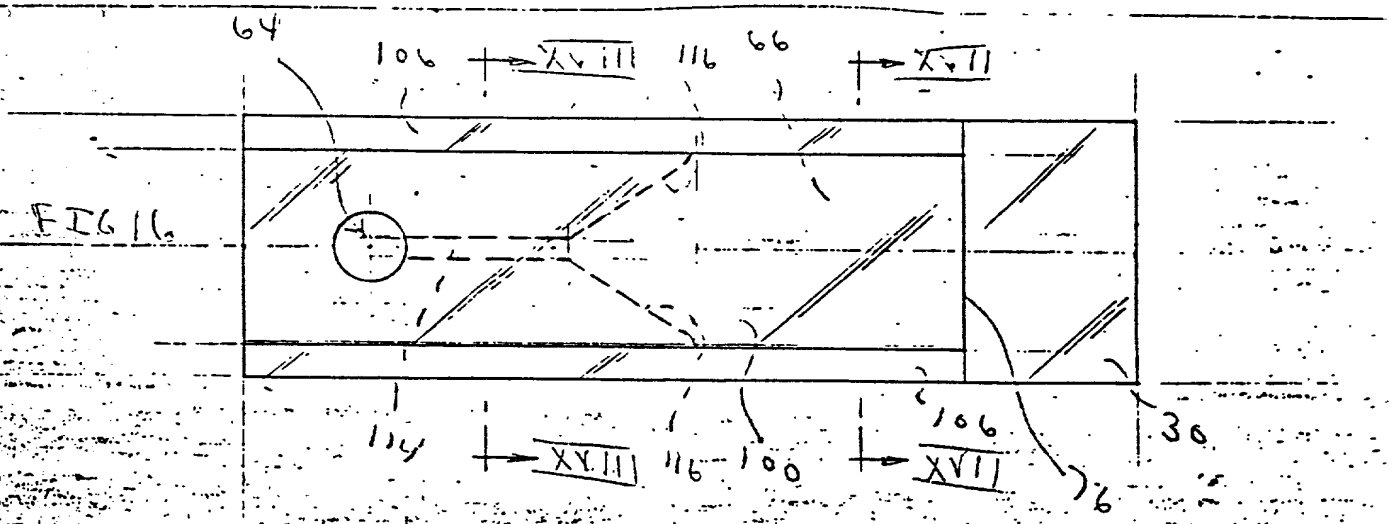


FIG 16

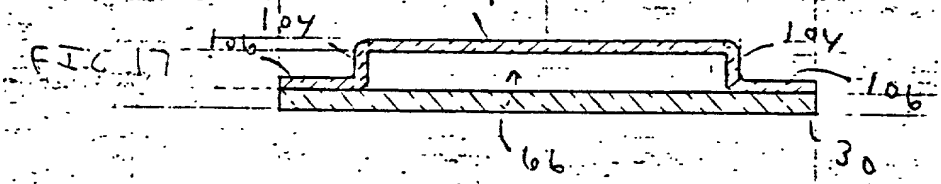


FIG 17

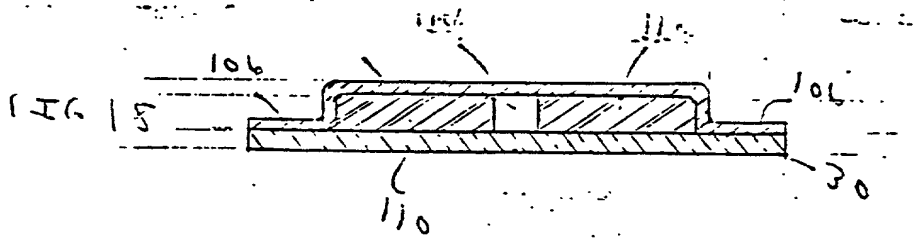


FIG 15

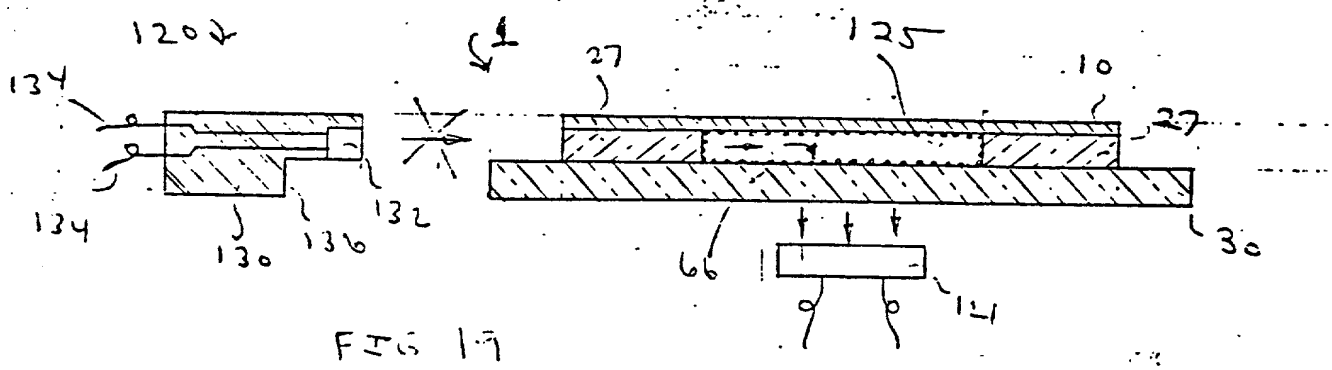


FIG 19

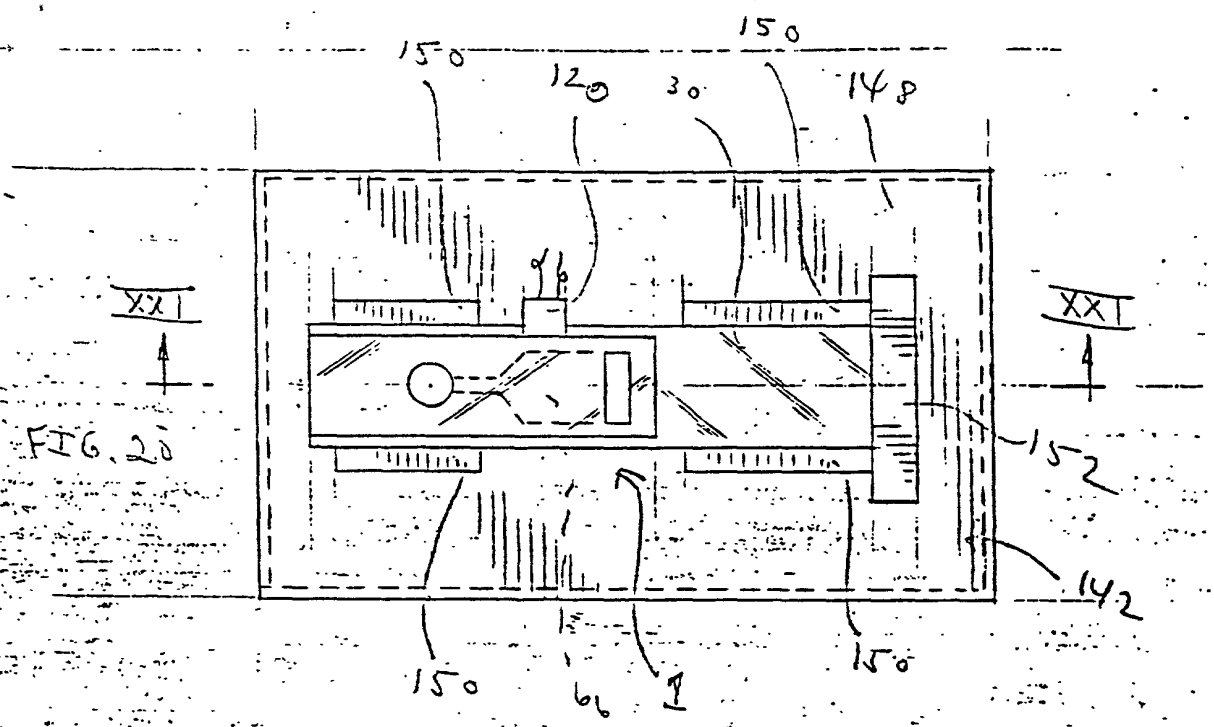

150
150
120
30
148
XXI
XXI
152
142
150
150
66
1

FIG. 2D

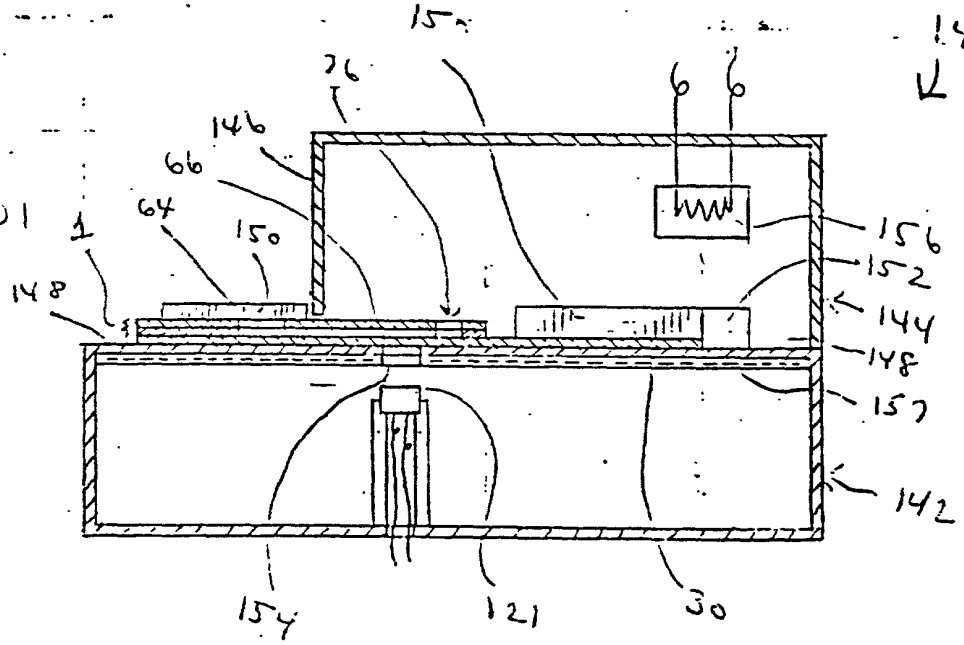
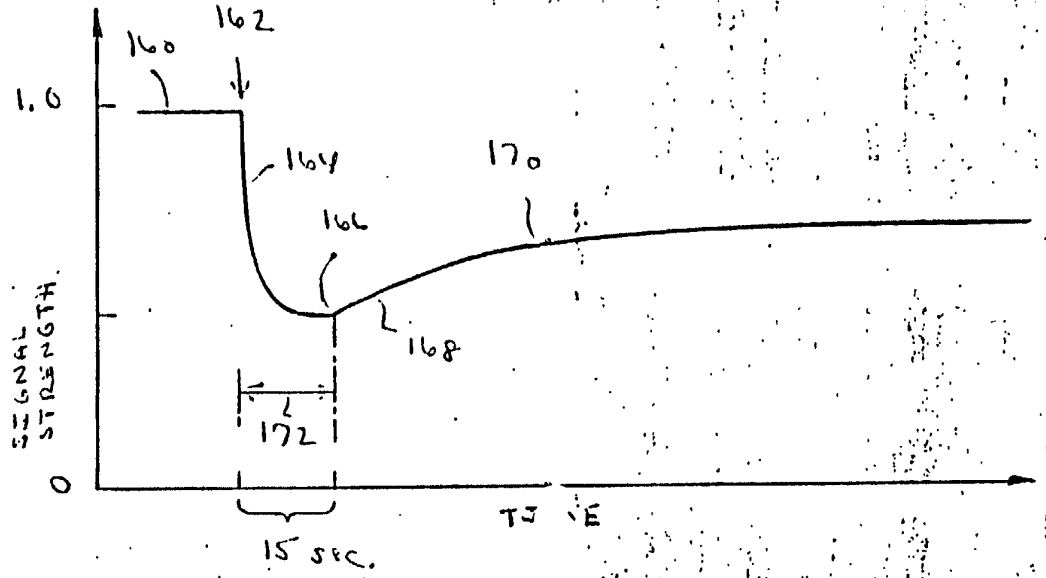

76
146
66
64
150
148
1
156
152
144
148
157
142
154
121
30

FIG. 3D

FIG. 22



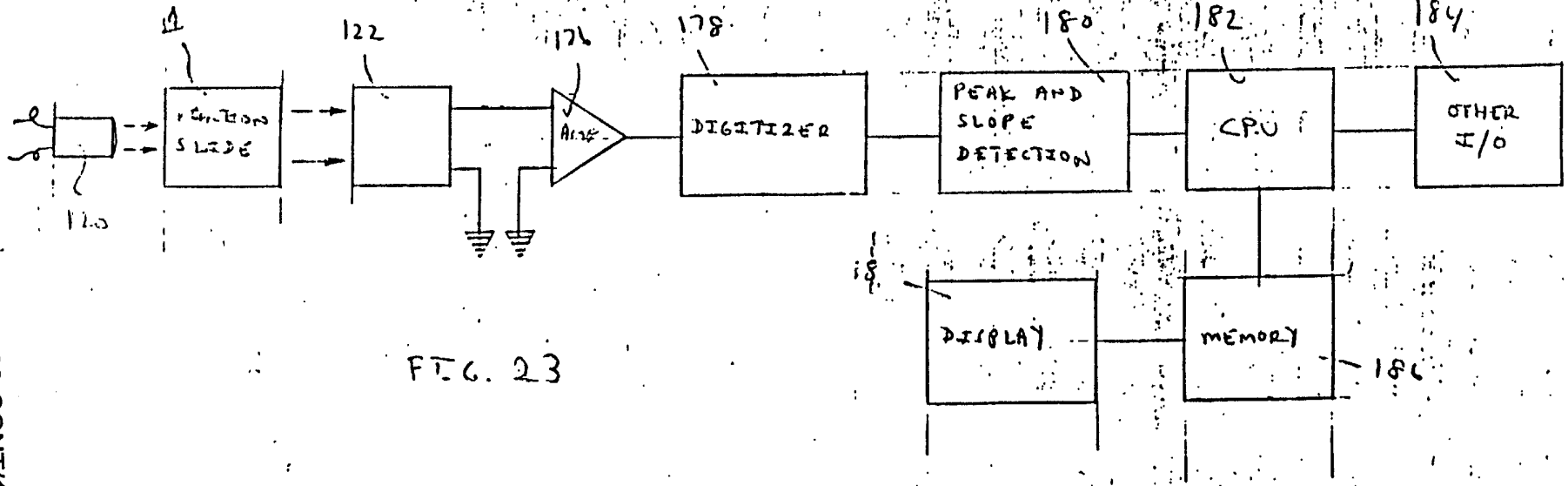


FIG. 23

033-CONT/10

FIGURE 63 is a top view of a reaction slide of the parallel flow type having three reaction spaces.

FIGURE 64 is a top view of a reaction slide of the serial flow type having three reaction spaces.

FIGURE 65 is a top view of a reaction slide of the parallel flow type having two reaction spaces, also showing light sources and detectors.

FIGURE 66 is a transverse elevational cross-section taken on line LXVI-LXVI of FIGURE 65. Together, FIGURES 65 and 66 illustrate apparatus that may be used in conducting a Plasminogen Activator assay.

FIGURE 67 is a longitudinal cross-sectional elevation of a reaction slide having a vent cover useful in selective venting.

FIGURE 68 is a top view of a reaction slide provided with a pinch valve.

FIGURE 69 is a section taken on line LXIX-LXIX of FIGURE 68, also showing a push rod for actuating the pinch valve.

FIGURE 70 is a top view of a reaction slide having an auxiliary conduit.

FIGURE 71 is a schematic representation illustrating how two liquids may be selectively introduced into a common chamber.

FIGURES 72-74 schematically illustrate various forms of cascading.

FIGURE 75 is a longitudinal vertical cross-section of a reaction slide together with apparatus for using suspended magnetic particles to measure a coagulation reaction.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Shown in FIGURE 1 is a top view of a cover 10 of a first embodiment of a reaction slide according to the current invention. Shown in FIGURE 2 is a top view of an overlay 20 of the first embodiment. Shown in FIGURE 3 is a top view of a base 30 of the first embodiment.

FIGURE 4 is an exploded view showing the relative positions of the cover 10, overlay 20 and base 30.

FIGURE 5 is a top view of the cover 10, overlay 20 and base 30, when assembled.

FIGURE 6 is a longitudinal vertical cross-section of a first embodiment of a reaction slide 1 according to the current invention. The cover 10, overlay 20 and base 30 are sectioned along line VI-VI of FIGURE 5. As will be described more fully below, the reaction slide 1 contains certain elements in addition to those shown in FIGURES 1-5.

Now referring generally to FIGURES 1-6, the cover 10 comprises a thin glass or polymeric sheet, typically

transparent, having formed therein a sample receiving opening 14 and an elongate opening 12 proximate a distal end 16 of the cover.

The overlay 20 comprises a thin glass or polymeric sheet, typically transparent, having formed therein a cut-out, the cut-out having a geometry as shown to form a sample receiving opening 22, a reaction space 24 and a conduit 26 communicating the reaction space and the sample receiving opening. (The reaction space 24 becomes a reaction volume upon assembly of the cover, overlay and base.) Advantageously, tapering walls 25 form a transition between the conduit 26 and reaction space 24. The distal end 28 of the overlay is closed as shown at 29.

The base 30 comprises a sheet of glass or polymeric material, typically transparent and typically somewhat thicker than either the cover 10 or overlay 20.

The cover 10 and base 30 are separated by a spacer 60 (FIGURE 6), the spacer 60 being made up of the overlay 20 sandwiched between two adhesive layers 62 which respectively join the overlay 20 to the cover 10 and the overlay 20 to the base 30. Each of the adhesive layers 62 has the same shape as the overlay 20. That is, each of the adhesive layers is formed with an opening having a shape corresponding to the

sample receiving opening 22, the reaction space 24 and the conduit 26 of the overlay 20. Accordingly, there are formed in the reaction slide a sample well 64, a reaction volume 66, a conduit communicating the reaction volume 66 and the sample well 64, and a vent 76 formed by the opening 12 in the cover 10 communicating the reaction volume 66 with the environment of the slide.

The bottom surface of the cover 10, facing the base 30, is spaced from the top surface of the base 30 by a distance that is sufficiently small to cause a sample placed in sample well 64 to be drawn into the reaction volume 66 by capillary action. Such action is made possible by the presence of the vent 76.

As shown in FIGURE 5, the length (left to right in the drawing) of the cover 10 is the same as that of the overlay 20, and the width (top to bottom in the drawing) of the cover 10 and overlay 20 are the same and are less than that of the base 30.

Preferably there is provided a liquid absorbing matrix (LAM) for withdrawing fluid from the reaction space when desired. To this end, there may be provided as shown in FIGURE 6 a LAM assembly 50 including a LAM pad 51, illustrated as a sponge, fixed on a LAM support 52, the LAM support 52 comprising an arm 53, a tab 54 fixed on the base 30, and a living hinge 55 joining the

arm 53 and tab 54. When the arm 53 is pressed downwardly, manually or by an automated presser (not shown), the LAM 51 will enter the vent 12 and make contact with fluid in the reaction volume 66, thereby drawing out the fluid. It has been found that, when the cover 10 is made of a polymeric material, this withdrawing action may be enhanced by downward deflection of a portion of the cover 10 adjacent the vent 12. It would appear that this enhanced withdrawal is caused by a localized narrowing of the distance between the cover 10 and base 30, thereby creating a narrowed passage to enhance capillary action. Thus although the cover 10 can be made of either rigid of flexible material, in this embodiment of the invention, the cover 10 is preferrably made of a flexible material.

Observations and measurements of chemical reaction occurring within the reaction volume 66 may be made by a number of methods, as described more fully below. At present, optical methods are preferred, but the choice of method will depend upon the assay being performed. Shown in FIGURE 4 are a number of paths that light may typically follow for making such measurements and observations. These paths may be used alone or in combination.

In light path 40, light is introduced through a side of the overlay 20 and passes initially through a portion of the overlay disposed between the closed end 29 and tapering wall 25. This portion of the overlay and its opposite corresponding portion will be referred to as internal waveguides 27. Thereafter, the light passes through the reaction volume 66 and out through the opposite waveguide 27. As illustrated schematically, light passing in this direction through the waveguides is internally reflected off the top and bottom surfaces of the overlay 20. Light path 40 is useful in making measurements based on the transmission or absorbance of light by the fluid within the reaction volume 66, in which case there is measured the ratio of light intensity before and after passing through the sample in the absence of scattering or excluding scattering. The Beer-Lambert Law describes the phenomenon. Standard detectors are employed in a line of sight configuration with the light source.

Light path 41 illustrates a measurement that may be made based upon light scattering in which light is first introduced transversly through an internal waveguide 27, enters the reaction volume 66, is then scattered by the sample, a portion of the scattered light proceeding downwardly through the base 30 and then leaving the reaction slide. Light scattering

measurements or nephelometry measures light which is not irreversibly absorbed by the sample and emerges at various angles, the spatial/intensity distribution being dependent upon particle size, shape and wavelength of the excitation energy. Rayleigh and Mie theories are useful models. Standard detectors are employed. Examples are photocells or photomultipliers, the latter being employed at very low light levels. Excitation source wavelength may be fixed at a particular value. The detector is typically set at a predetermined angle from the direction of excitation.

Light paths 42 and 43 respectively show light entering laterally through the sides of the cover 10 and base 30, experiencing total internal reflection as it passes directly above and beneath the reaction volume, respectively, and exiting through the opposite edge of the cover or base. As will be explained more fully below, such light paths may be employed for detecting fluorescence using an evanescent wave measurement.

Other light paths are possible, including vertical paths passing through the cover, reaction volume and base and light paths making use of reflectance off a sample in the reaction volume, according to which light may both enter and leave the reaction volume by way of the cover 10 or base 30.

It will typically be desirable to exclude stray light from entering the reaction slide. For this purpose, any external surface of the reaction slide which is not to be used for the transmission of light may desirably be painted with an opaque paint. The choice of surfaces to be so painted will be governed by the assay to be performed and the elected methods of measurement. When any of the components 10, 20 or 30 will not be used for the transmission of light, that component may be made of a material which is itself opaque, such as metal.

When using light paths such as 40 and 41 in FIGURE 4, it becomes important to transmit as much light as possible through one or both of the internal waveguides 27, keeping the losses as low as possible. It has been found that the presence of the adhesive layers 62 can cause the spacer 60 to perform like an optical fiber, the waveguides 27 corresponding to a core of an optical fiber and the adhesive layers 62 corresponding to cladding.

Refractive index mismatch between the waveguide 27 and the adhesive layers 62 produces total internal reflection of light striking the interface at angles greater than the critical angle. By way of example, reference is made to FIGURE 24, wherein there are shown core 70, cladding 71 surrounding the core 70, and

incident light ray 72 striking and passing through the core 70. The core 70 may correspond to the internal waveguide 27 of the overlay, and the cladding 71 may correspond to the adhesive layers 62. If, for example, the core material 70 has a refractive index n_1 of 1.62 and the cladding 71 has a refractive index n_2 of 1.52, the sine of the critical angle is n_2/n_1, or 1.521/1.62 = 0.938. The critical angle is then 69.8 degrees.

Referring now to FIGURE 7, there is shown a fragmentary vertical cross-section of a modification of the embodiment of FIGURES 1-6, the view of FIGURE 7 being taken at a representative location corresponding, for example, to the extreme left-hand portion of FIGURE 6. In this modification, the spacer 60 includes a second overlay 68 which is substantially identical to the overlay 20. A third adhesive layer 62 is used to join the overlay 20 and the second overlay 68. Because the second overlay 68 is identical to the first overlay 20, it forms a second pair of internal waveguides 27. The additional cross-sectional area provided by the additional waveguides 27 substantially increases the amount of light that may be introduced into the reaction volume 66 through the internal waveguides 27.

Referring now to FIGURE 8, there is shown a top view of a second embodiment of a reaction slide 1 according to the current invention. The base 30 and

overlay 20 of this embodiment are identical to those shown in the embodiment of FIGURES 1-6. However, the cover 10 has a length that is less than the length of the overlay 20. There is provided an end cover 75, coplanar with the cover 10 and spaced therefrom to form a gap. This gap creates vent 76, communicating the reaction volume 66 with the environment of the reaction slide 1. For the sake of clarity, the LAM assembly 50 is not shown.

Additional variations of the above-described embodiments are possible. For example, it is not necessary for a LAM assembly to be fixed on the base 30. Such an assembly may be provided separately and may be manipulated manually or using an automated system.

The adhesive layers 62 may be omitted, and an alternative method such as heat sealing may be used to join the cover 10, overlay 20 and base 30. In such a case, the spacer 60 is formed entirely by the overlay 20. Referring again to FIGURE 24, in such a case the internal waveguide 27 of the overlay 20 will again act as a core 70 of an optical fiber, but the cladding 71 will be formed by the cover 10 and base 30.

FIGURES 9-11 show top views of, respectively, a cover 10, overlay 20 and base 30 of a third embodiment of a reaction slide according to the current

invention. FIGURE 12 shows a top view of the assembled reaction slide of this embodiment. This embodiment differs from that of FIGURES 1-6 in the omission of conduit 26 and in that the distal end 28 of the overlay 20 is open, so that the reaction space vents longitudinally between the cover 10 and base 30 instead of vertically through the cover.

The cover 10, overlay 20 and base 30 may be secured to each other using adhesive layers 62 as described in connection with the previous embodiments, or the adhesive layers 62 may be omitted and the various elements of the reaction slide may be joined by heat sealing or solvent bonding, etc.... It has been found that, where heat sealing is employed, discontinuities in the heat seal often result, impairing the total internal reflection of light when passing through the waveguides 27. To compensate for such impairment, a reflective layer 78 may be placed atop the waveguide 27 of the overlay 20 through which light will be introduced. A corresponding reflective layer 80 may be placed on the base 30. Such reflecting layers also may be used in other embodiments according to the current invention, if desired.

FIGURE 13 illustrates the open-ended reaction volume of the embodiment of FIGURES 9-12, together with the addition of a LAM 82 in a configuration preferred

for use with such an open-ended reaction volume. In particular, the LAM 82 is fixed on the base 30 and overhangs the distal end 16 of the cover 10. When it is desired to remove liquid from the reaction volume 66, the LAM 82 is depressed, resulting in a localized deformation of cover 10 and LAM 82, causing contact between the LAM 82 and the fluid in the reaction volume 66 for the withdrawal of the fluid.

Shown in FIGURE 14 is a top view of a fourth embodiment of a reaction slide 1 according to the current invention. In this embodiment, the distal end 28 of the overlay is open, as is the case with the embodiment of FIGURES 9-13, such that the reaction volume 66 vents between the cover 10 and base 30. The cover 10 is shorter than the overlay 20, so that a portion of the overlay 20 may be seen extending to the right in the drawing from beneath the cover. The overlay 20 is provided with a first conduit 90 communicating the sample well 64 with the reaction volume 66 and a second conduit 92 extending backward to a point beyond the sample well, such that the end portion 94 of conduit 92 extends beyond the edge of the cover. The second conduit 92 and its end portion 94 are used for visual inspection to determine that proper filling has been achieved.

In particular, in a typical use of a reaction slide according to the current invention, that portion of the reaction slide containing the reaction volume 66 will be disposed within a measuring instrument, whereas that portion of the reaction slide containing the sample well will extend out of the measuring instrument so that a sample may be introduced into the sample well 64 when desired. When the sample passes from the sample well 64 into the reaction volume 66, it no longer is visible to the user, that portion of the reaction slide being disposed within the measuring instrument. Accordingly, when the user observes the presence of sample in end portion 94 of second conduit 92, it is assured that proper filling has been achieved. It may be seen that a reaction slide according to this embodiment is most useful in those cases when the cover 10 is opaque. In the alternative, if the cover is transparent, substantially all of the second conduit 92 may be used for visual observation of proper filling. In such a case, it is not necessary that the second conduit 92 extend beyond the end of cover 10 as illustrated, and the length of the cover 10 may be the same as the length of the overlay 20.

Second conduit 92 is not essential, as proper filling may be monitored by electro-optic means using the same light detectors used in monitoring the results

of the assay being performed. Indeed, the embodiments of FIGURES 1-13 do not employ a second conduit 92.

FIGURE 15 is an exploded view of a fifth embodiment of a reaction slide 1 according to the current invention. A top view of this embodiment is shown in FIGURE 16, with selected vertical transverse cross-sections being shown in FIGURES 17 and 18.

There is shown a base 30 on which is fixed an insert 110 and insert cover 100. Insert cover 100 is generally formed by a major planar segment 101 having lateral sides bent downwardly outward to form walls 104 and then laterally to form tabs 106. The tabs 106 are bonded to the base 30 with the insert 110 being disposed between the planar segment 101 of the insert cover 100 and the base 30, the height of the walls 104 generally corresponding to the height of the insert 110.

Insert 110 includes a sample receiving opening 112 communicating with a conduit 114 which ends in outwardly tapering walls 116. As the length of the insert 110 is substantially less than the length of the insert cover 100, a reaction volume 66 is formed to the right of the insert as shown in FIGURE 16.

Thus, it may be seen that the walls 104 in the area of the reaction volume 66 serve the functions of the internal waveguides of the previous embodiments,

and for this purpose at least those portions of the walls 104 that are disposed in the vicinity of the reaction volume 66 are made of a transparent material.

Although the insert 110 may be bonded to the base 30, variations are possible. For example, the insert and base may be formed as one piece, molded or machined to the appropriate shape and channel structure.

As will be described in more detail below, an assembled reaction slide according to the various embodiments will typically contain one or more reagents specifically selected for their utility in performing any of the many assays that may be performed using reaction slides according to the current invention. For example, liquid reagent may be placed in the reaction volume by filling through the sample well or, preferably, through the vent. The reagent can then be freeze-dried, the exact conditions of the freeze-drying process being dependent upon required optima and the type of reagent employed. There is thus produced a reaction slide, ready for use, having a premeasured amount of reagent disposed therein.

Typically, it may be desired to modify the internal surfaces of the reaction slide which will contact the sample or reagent or both to modify the liquid/solid/air contact angle of the surfaces, the surfaces thus being treated to increase their

hydrophilic character. Such treatment will increase the ease with which the sample flows from the sample well to the reaction volume.

There are a variety of methods available for decreasing contact angle on a hydrophobic (or nonpolar) surface, thereby rendering it more hydrophilic. Surface active agents (or surfactants) which are typically employed as wetting agents may be used. For example, small amounts of Triton type dispersion agents, Tween (polyoxyethylene derivatives of fatty acid partial esters of hexitol anhydrides) type surface active agents, and Brij (polyoxyethylene ethers of higher aliphatic alcohols) type wetting agents may be utilized. Surface modification via chemical derivitization of surface molecules can create polar prosthetic groups. Other techniques include surface modification using controlled electrical discharge or plasma treatment.

It should be noted that the height of the reaction volume is critical and is defined by the thickness of the spacer 60. This height should be uniform and can range from 0.001 to 0.02 inches (approximately). Typically, this height is preferably from 0.002 inches to 0.008 inches, and most preferably approximately 0.006 inches.

This order of magnitude is not only appropriate to achieve functional capillary action in the channels but is of the same order of magnitude as is required, generally, for optical waveguide transmission of light by total internal reflection. Coincidentally, this dimension is approximately of the order of magnitude required to produce preferential phase separation to the center of a flowing stream of suspended particulate or cellular material in a two phase system (or suspension) during sustained laminar flow conditions which may be achieved, as will be described below.

For construction of the reaction slide, all materials which come in contact with sample or reagent should be relatively inert. The surface properties of the materials should be such that appropriate wetting of the surface is achieved by the sample to provide proper flow conditions. Generally a low contact angle is best.

Cover 10 may be fabricated from a solid thin sheet of paramagnetic material or a laminate consisting of a coated paramagnetic material or could be fabricated from plastic or glass.

The paramagnetic material could be iron or nickel. Chemically inert thin coatings, such as polyvinyl chloride, acrylic, or polycarbonate could be utilized. A polymer with encapsulated iron oxide (e.g., magnetite) could be utilized as well.

The cover also could be fabricatd from a variety of glasses and fused quartz. Polymeric materials which could be advantageously utilized include: polycarbonate, PET, PETG (glycol-modified polyethylene terephthalate), acetate, acrylonitrile, and cellulose nitrate. A variety of coextruded films, composites and polymer alloys may also be used. Of primary importance are diminsional stability, stiffness, resiliency, and optical clarity (when required). The ability of a material to be fabricated in thin sheets is also a factor. Methyl methacrylate and polystyrene are both potentially suitable materials but are difficult to fabricate in thin sheets.

The cover is typically of greater surface area (or projected area) than the reaction volume. The cover may typically assume the same length and width as the spacer (e.g., 2 inches x. 0.5 inches) but could be larger, if required, or smaller.

Materials which may be utilized to produce a good to excellent overlay include: polycarbonate, PETG, methyl methacrylate, polystyrene and glass. However, glass is difficult to fabricate into the required shapes. Materials which may be utilized to produce a good to moderately good overlay include: polyvinyl chloride, nylon (polyamides), PET ~~or polyethylene~~ terephthalate (e.g. mylar), and acetate. Materials

which may be utilized to produce an acceptable overlay include: acrylonitrile, low density polyethylene film, PP/EVA coextruded film, EVA/nylon/EVA coextruded film, PP/EVA/PE/EVA coextruded film, and oriented polypropylene film. Materials which may produce an overlay of marginal acceptability include: XT and high density polyethylene film. In general, the better materials provide better waveguides because they have lower light scatter losses and transmit well in the visible spectrum where the most commonly employed excitation wavelengths may be found.

There are many adhesives which can be employed to secure the overlay to the cover and base. Acrylic adhesives are generally good. The best adhesives retain some flexibility, are transparent, and have low light scatter losses when cured, pressure treated, or otherwise activated. The length and width of the overlay may be varied over a wide range, but could be typically and approximately 2 inches x 0.5 inches on a 3 inch x 0.75 inch base. Thickness of the spacer is typically in the range of 0.002 to 0.010 inches. Thinner spacers may occasionally result in impeded capillary channel flow. Thicker spacers tend to lose liquid at the air interface adjacent to the edge of the reaction volume due to poor capillary action at larger diameter conduits.

The base is a solid support and can be made from a variety of materials. It should be rigid enough to maintain and support the reaction volume geometry, transparent in the reaction volume region (if required for monitoring) and capable of being bonded to the spacer/cover component. Fluorinated hydrocarbons such as Teflon make poor bases because they are difficult to bond. Glass is an acceptable material. Excellent bonding may be achieved with polycarbonate or methyl methacrylate base materials. A typical minimum thickness for the base is approximately 0.020 inches for a material such as polycarbhonate. An aluminum base (if transparency is not required) could be thinner. If the base is too thin, it may bend too easily and alter the volume of the reaction volume unintentionally during handling or manipulation during an assay. If the base is too thick, it may take too long to achieve thermal equilibrium for a temperature controlled assay. This is especially true for materials with low heat conductivities. The length and width of the base are variable. The base could be as small as 0.25 inches in width and 1 inch in length (or even smaller). Typically, the base will be approximately 0.75 inches in width and 3 inches in length. This provides enough room for a sample well, connecting conduit, and reaction volume with vented

FIG. 24

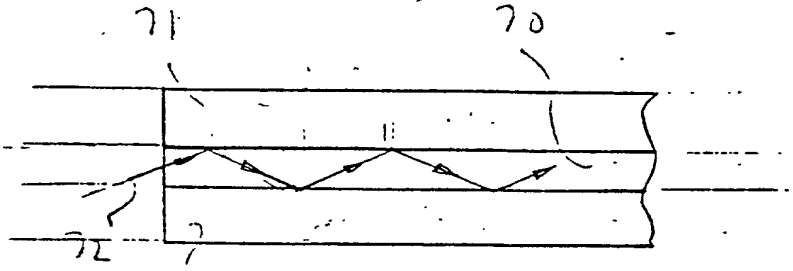


FIG. 25

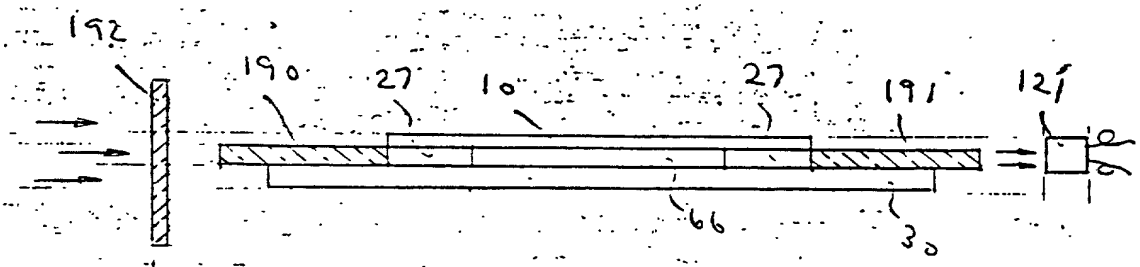


FIG. 26

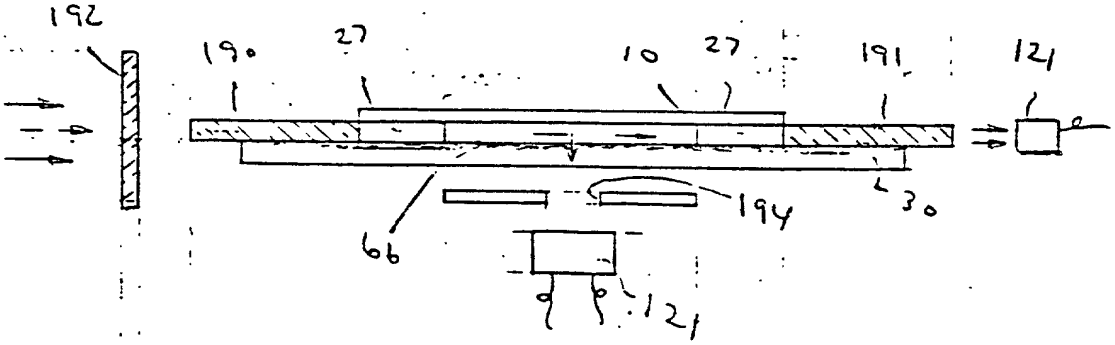


FIG. 27

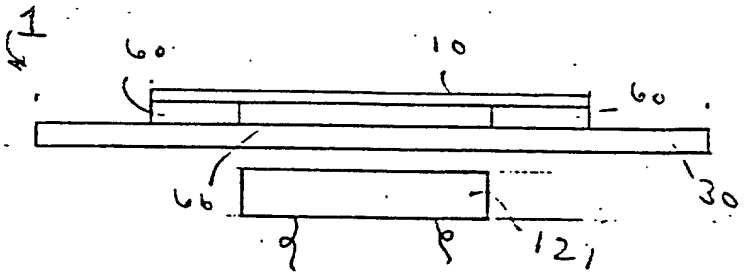
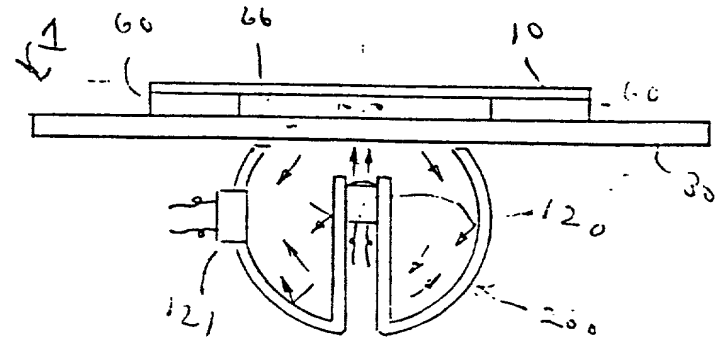


FIG. 28



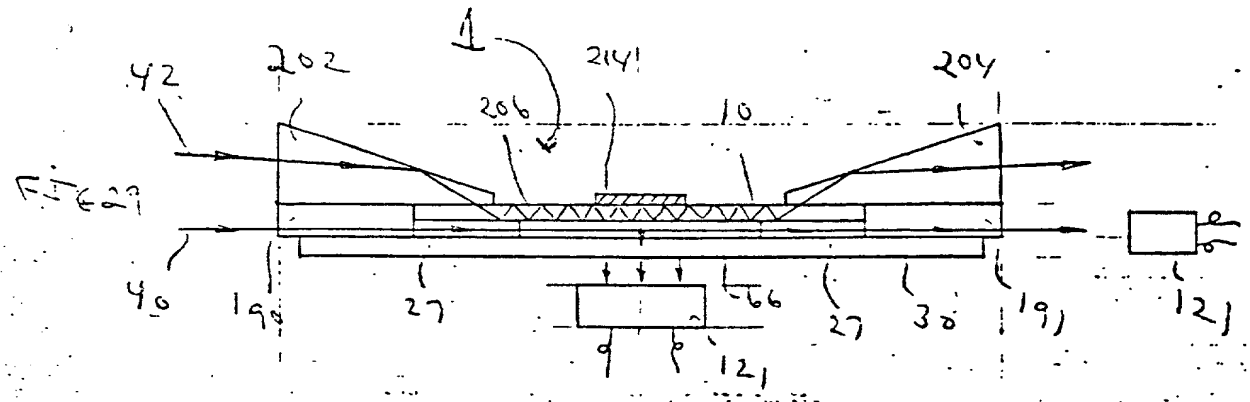


FIG 29

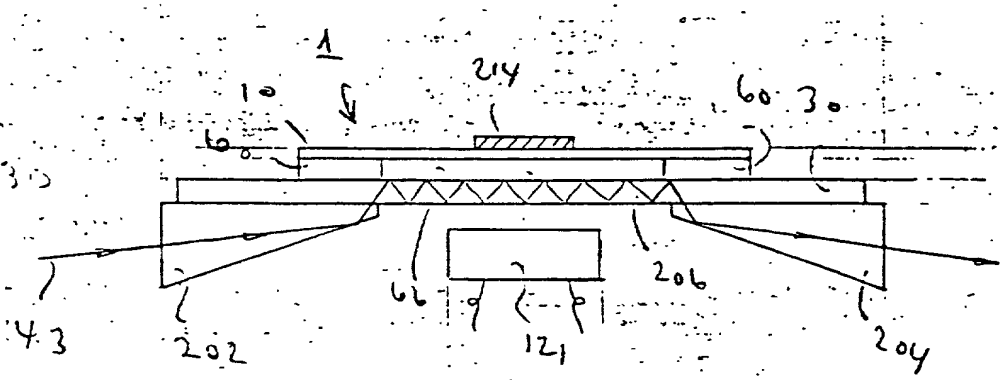


FIG 30

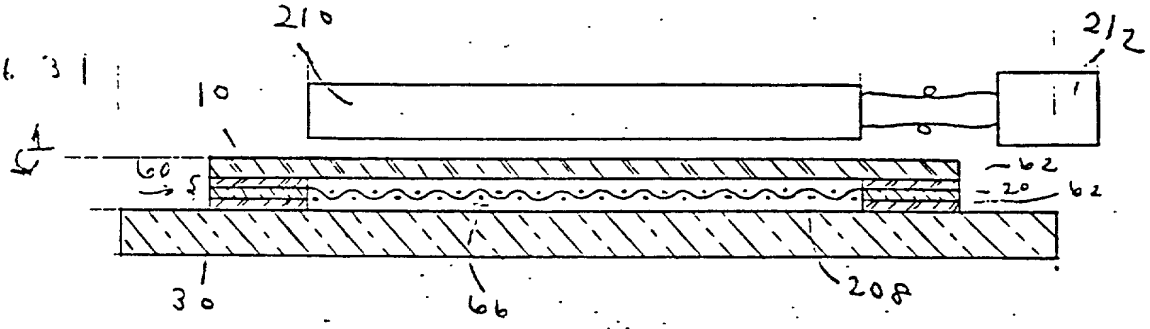


FIG 31

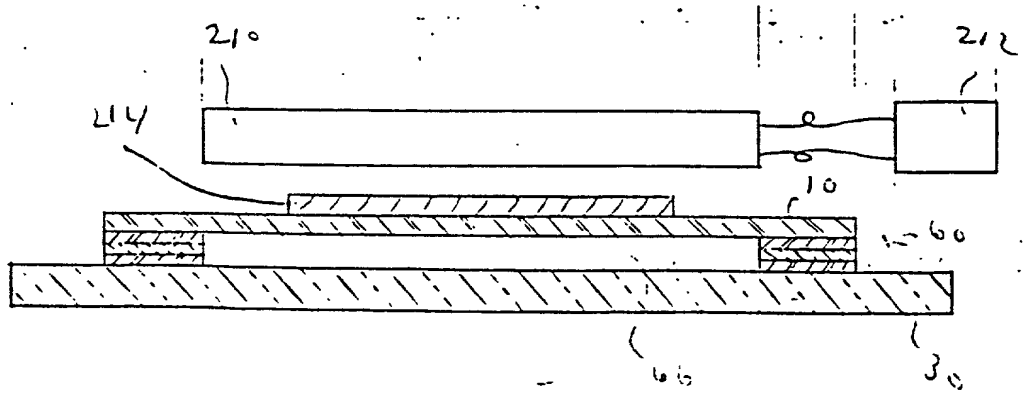


FIG 32

FIG. 33

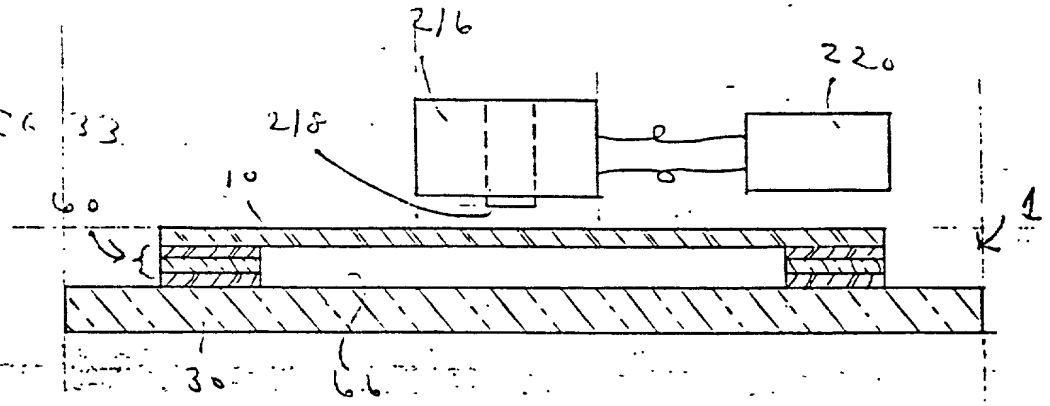


FIG. 34

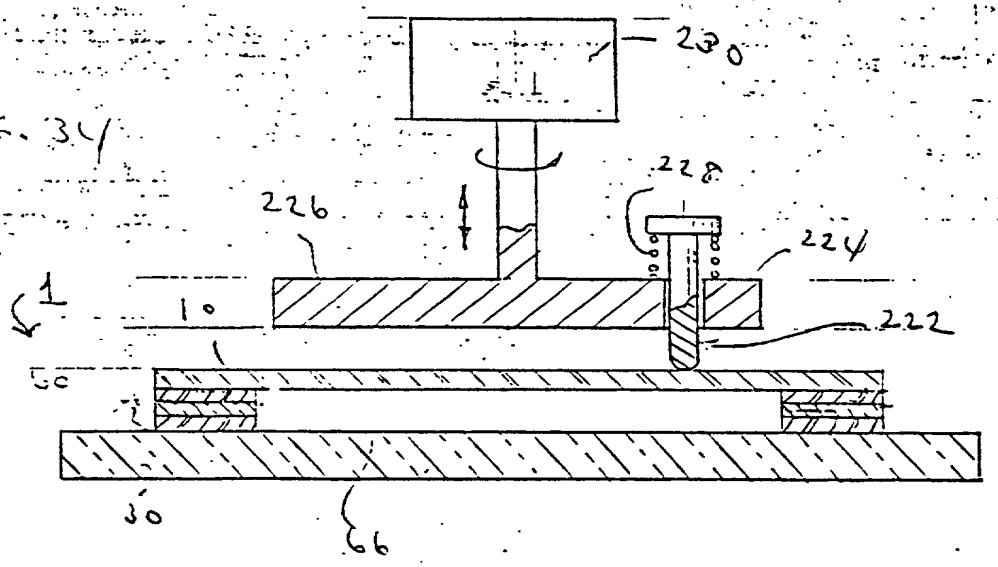


FIG. 35

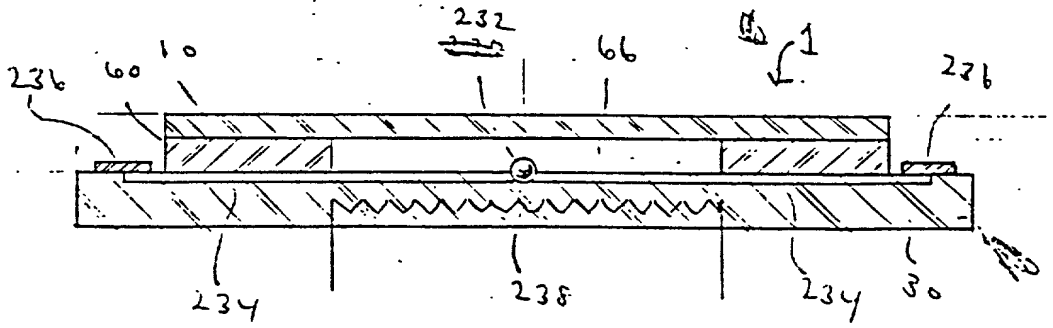
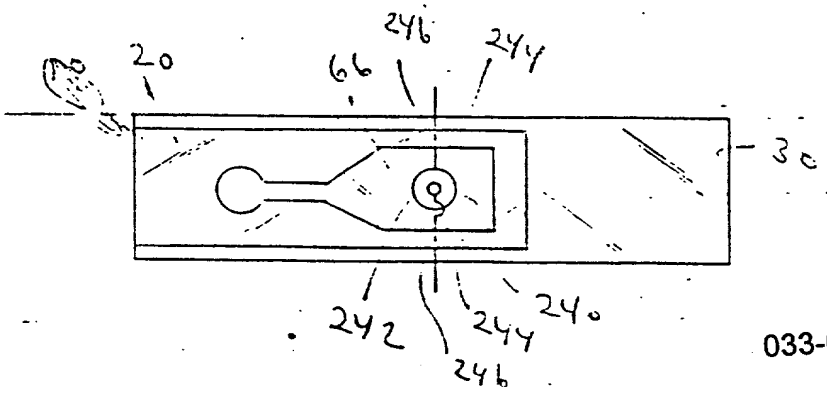


FIG. 36



033-CONT/13

FIG. 37

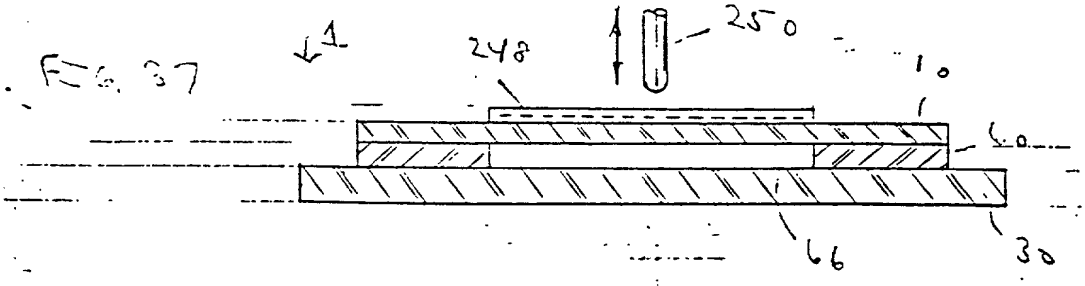
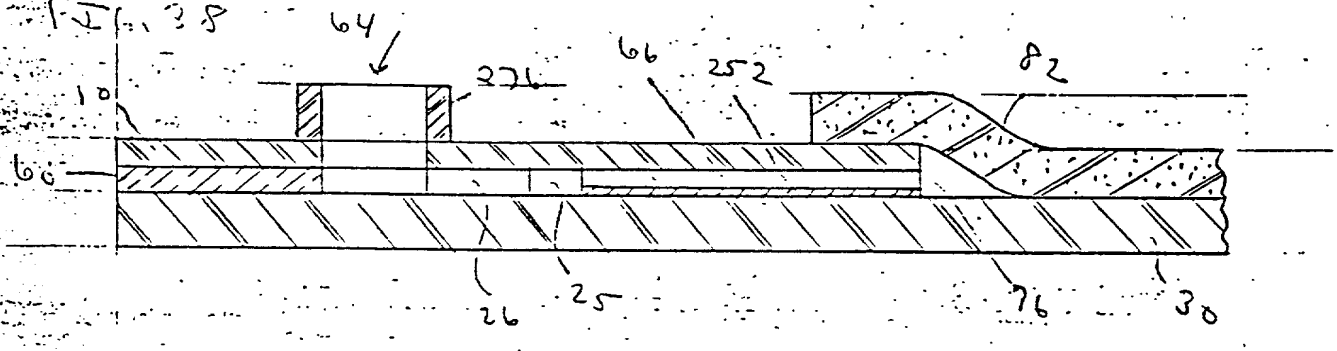


FIG. 38



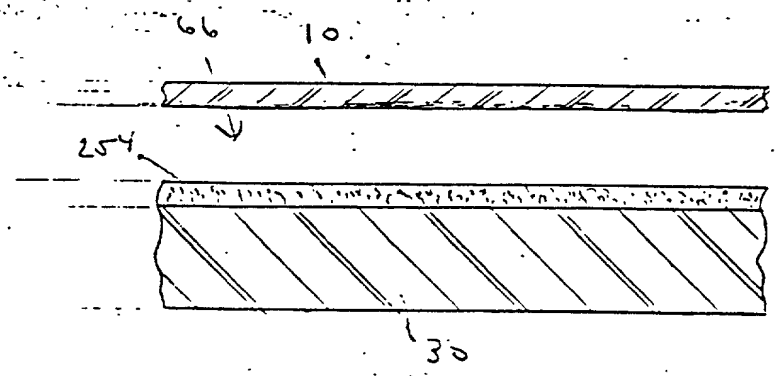


FIG. 39

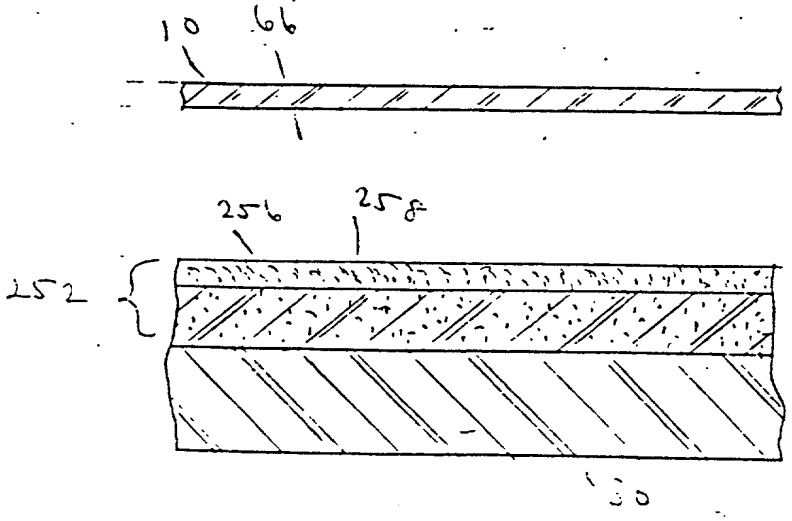


FIG. 40

FIG. 41

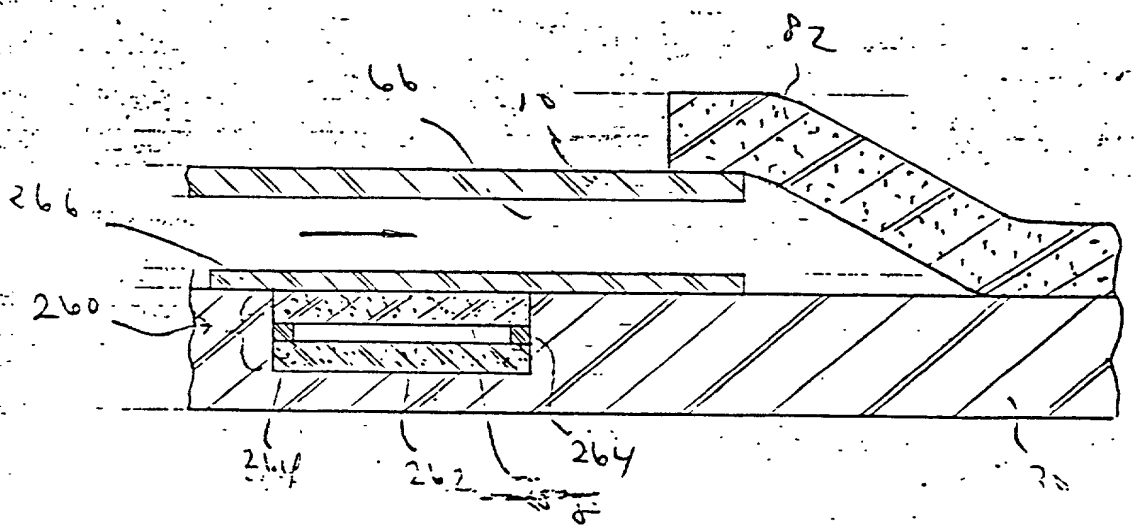
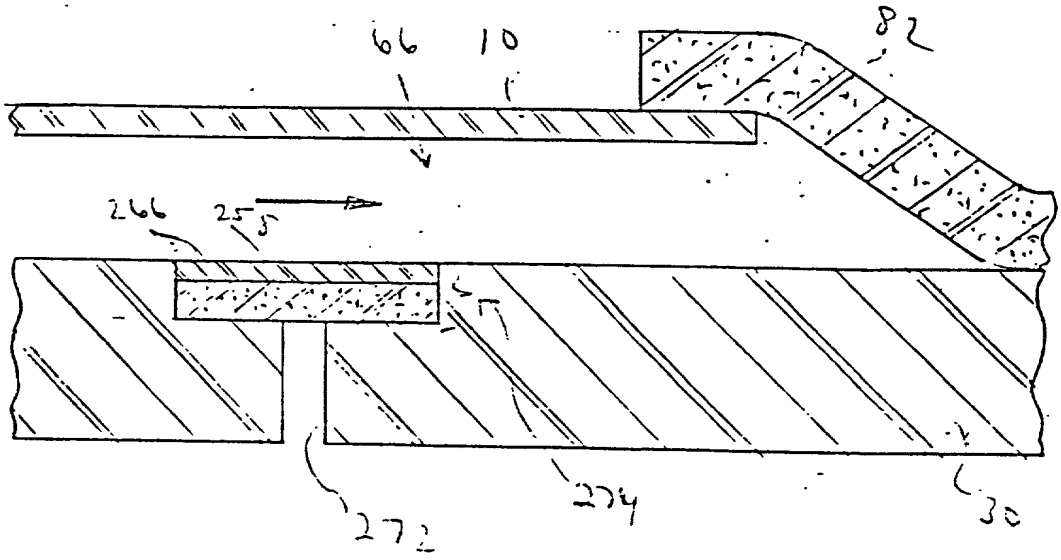


FIG. 42



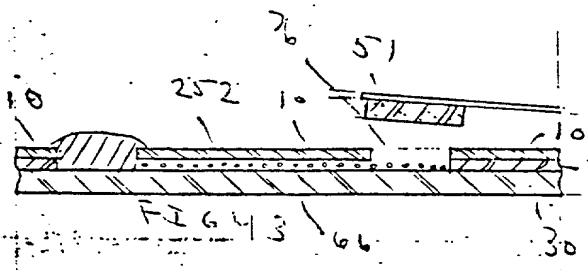


FIG 43

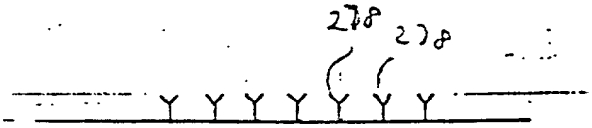


FIG 52

FIG 44

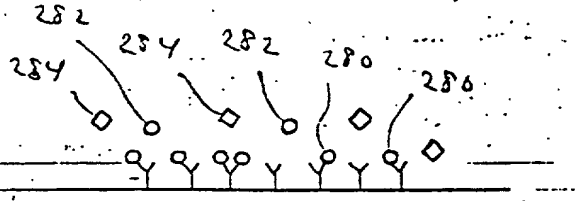


FIG 53

FIG 45

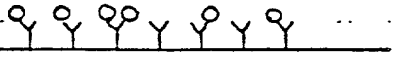


FIG 54

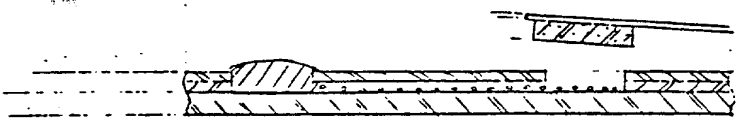


FIG 46

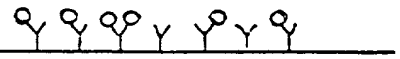


FIG 55

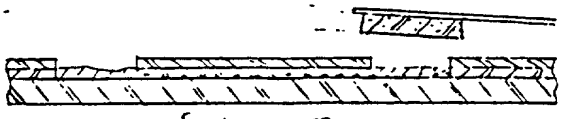


FIG 47

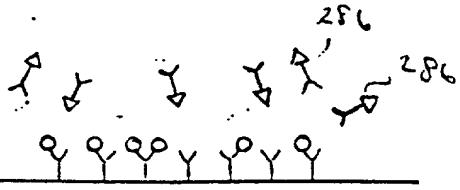


FIG 56

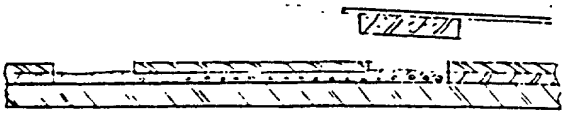


FIG 48

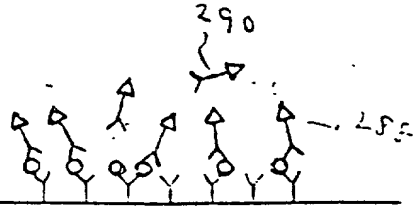


FIG 57

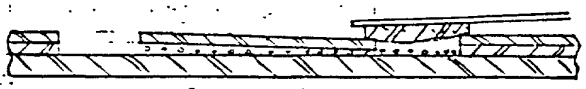


FIG. 4

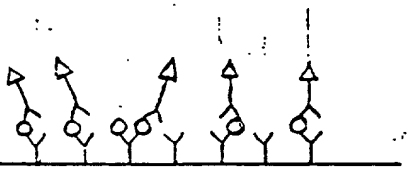


FIG. 4

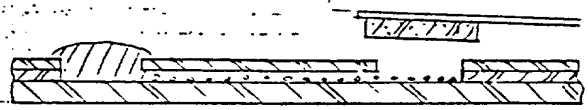


FIG. 5

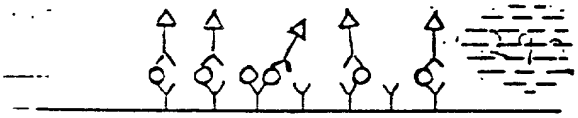


FIG. 5

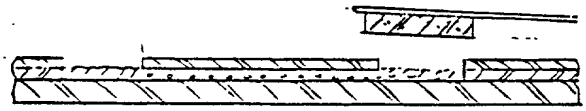


FIG. 6

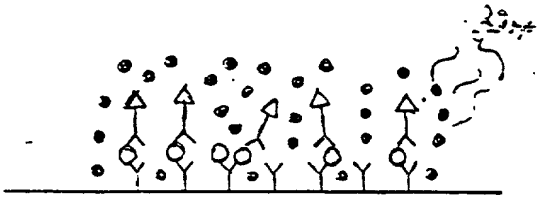


FIG. 6

033-CONT/17

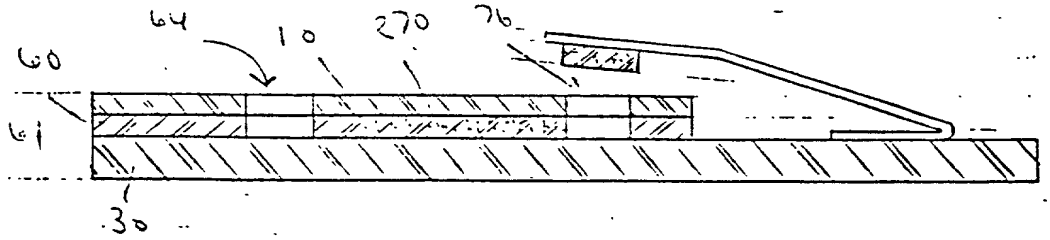


FIG 61

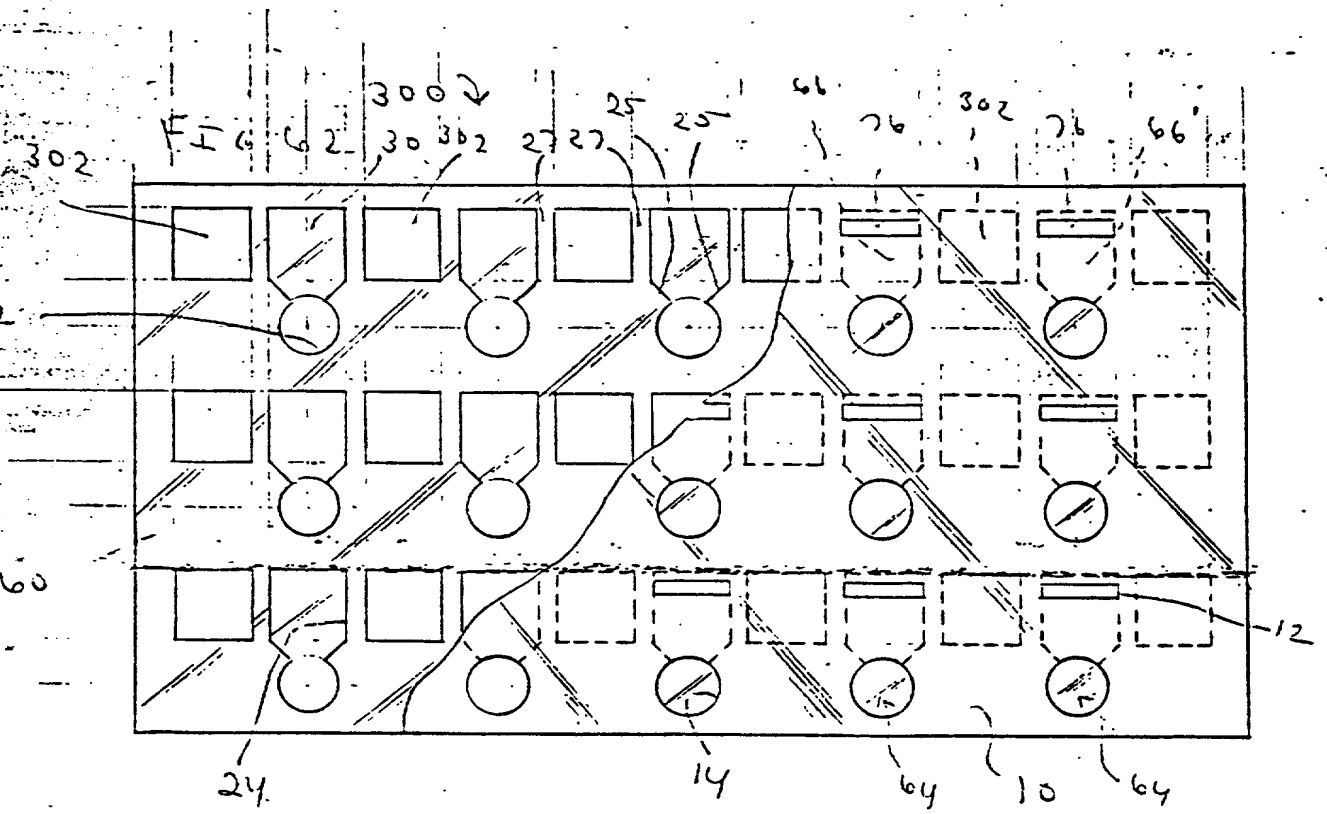


FIG 62

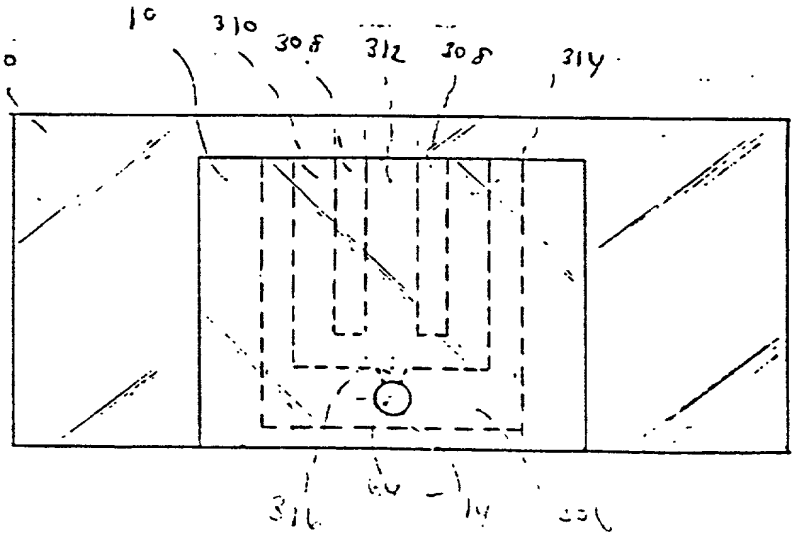


FIG 63

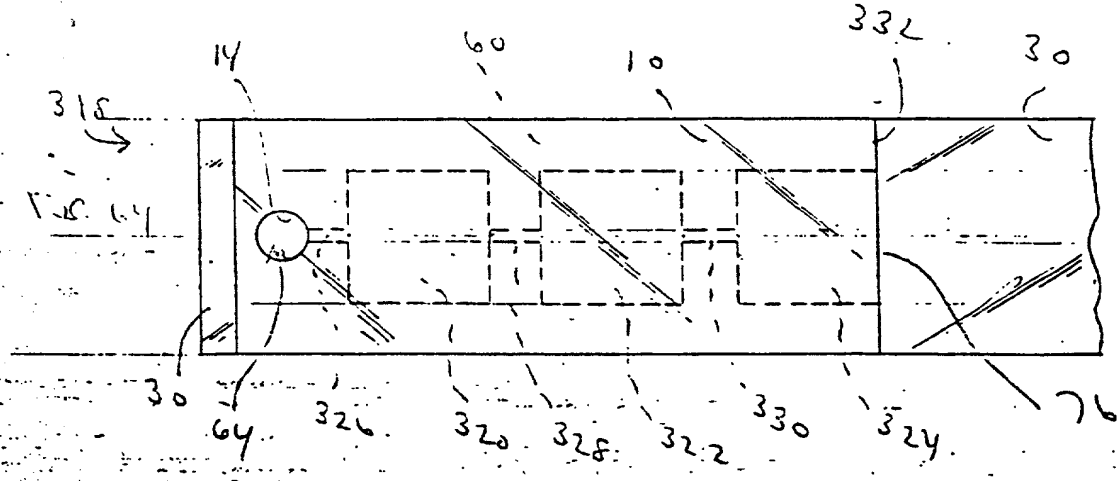


FIG. 64

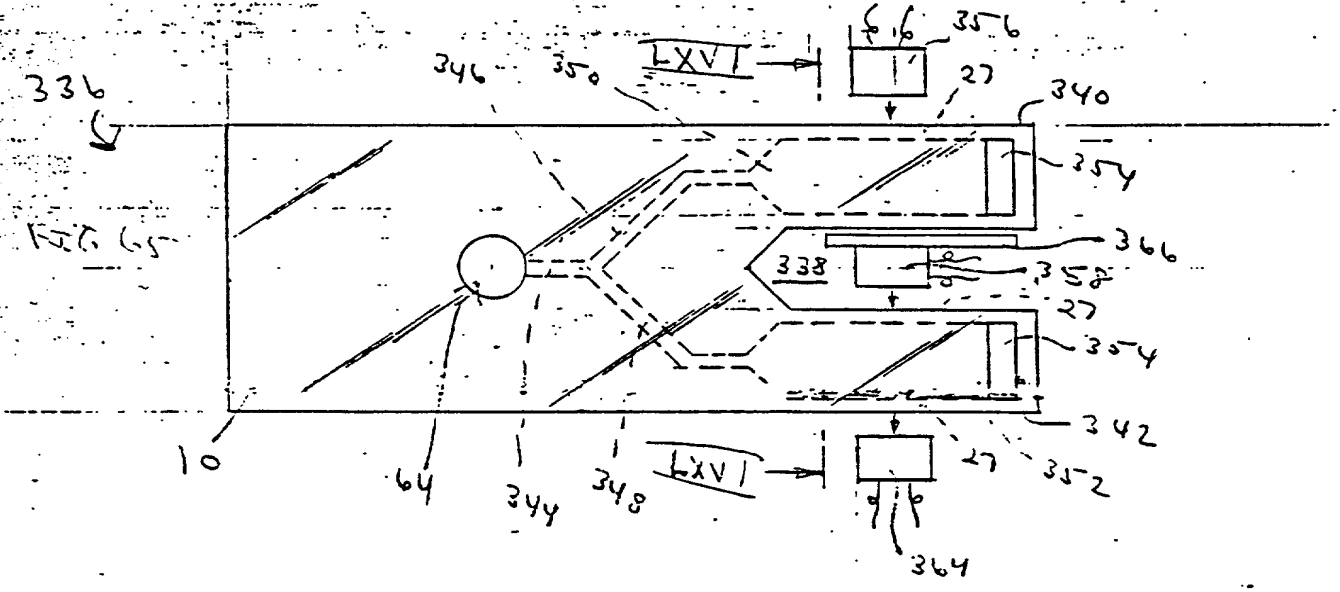


FIG. 65

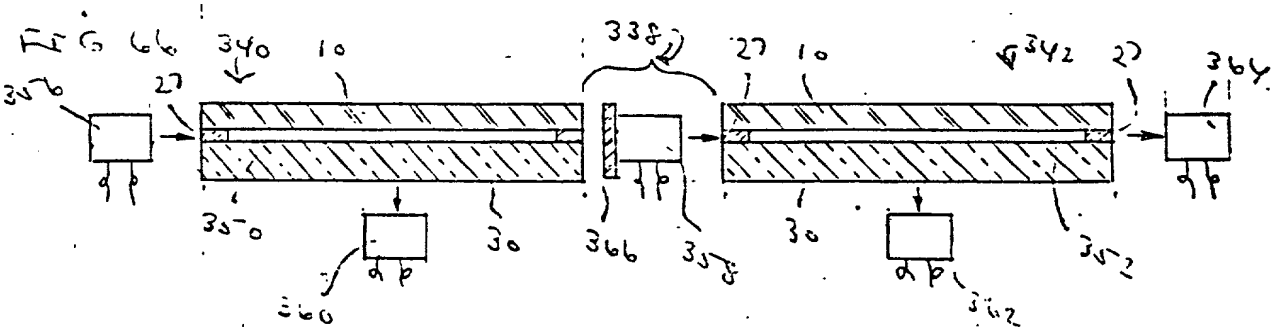


FIG. 66

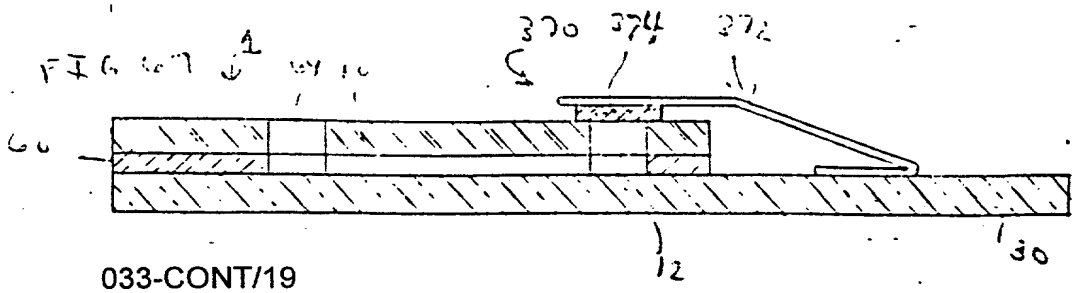


FIG. 67

033-CONT/19

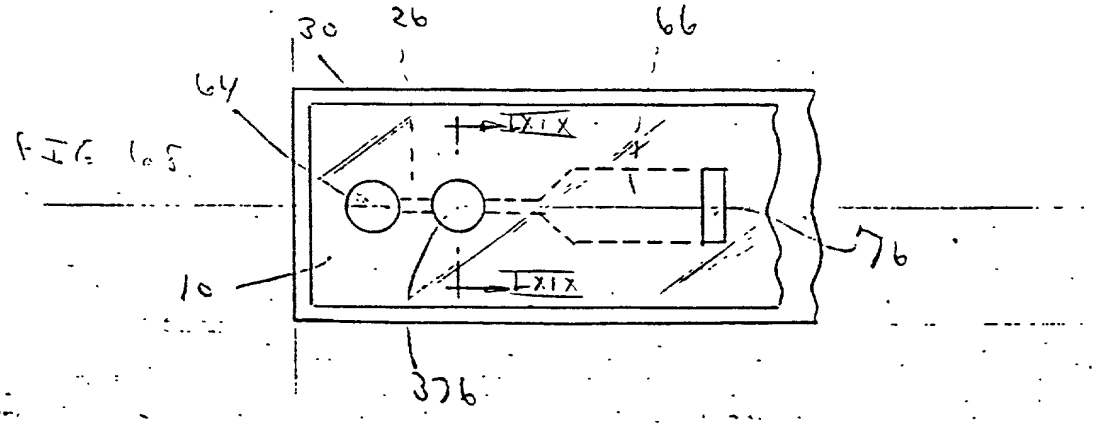


FIG 69

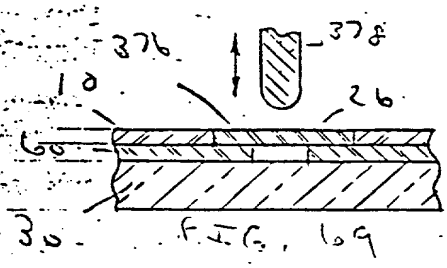


FIG. 69

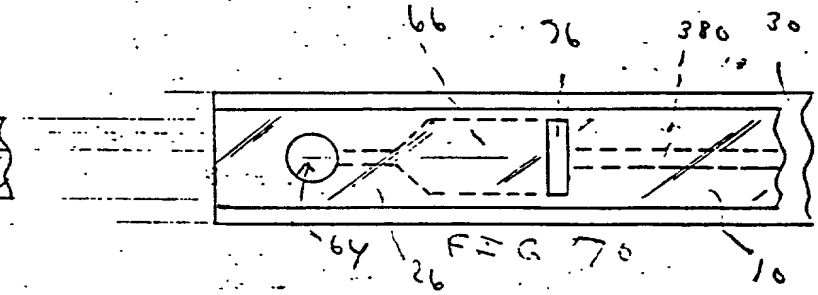


FIG 70

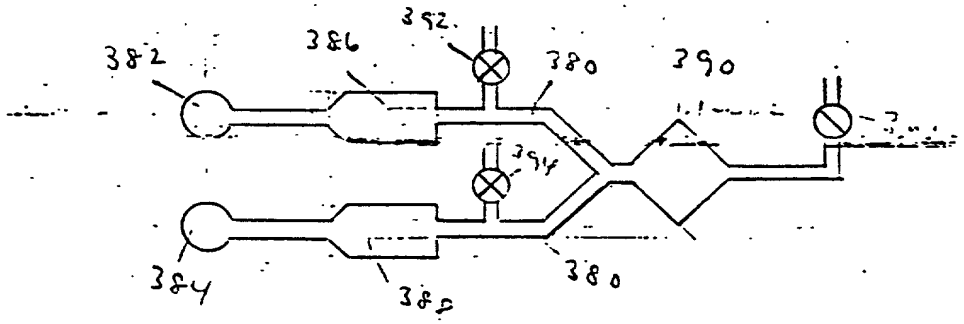
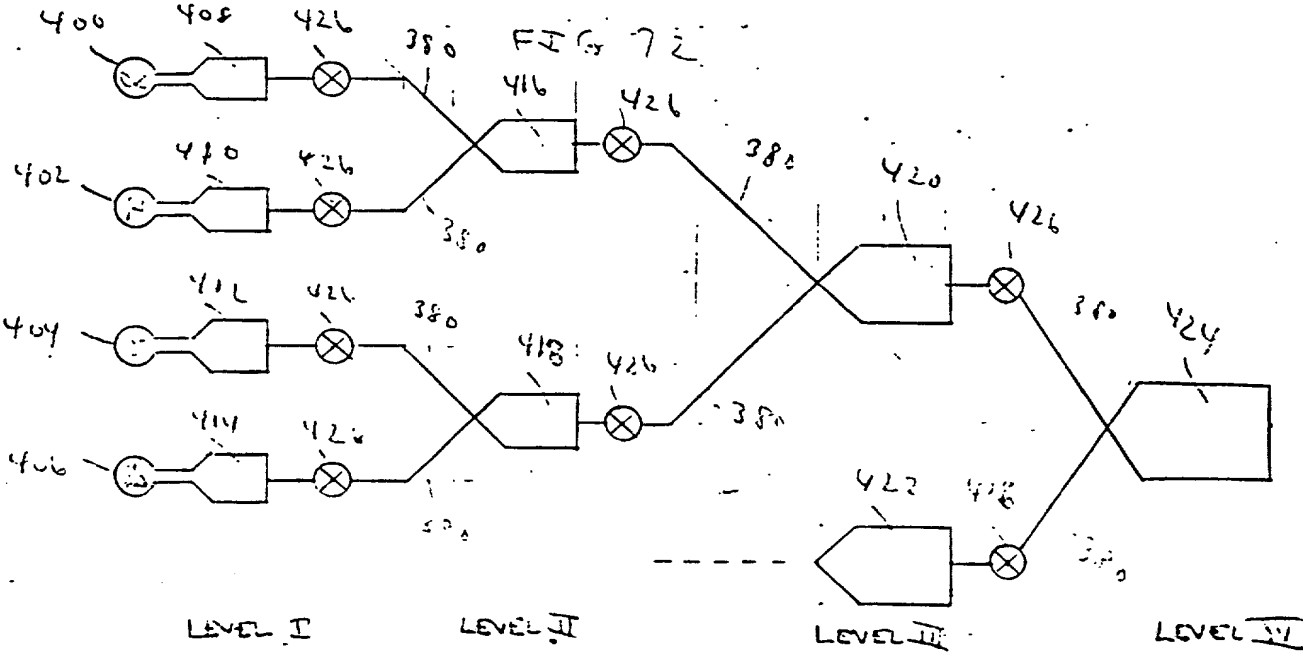


FIG 71



033-CONT/20

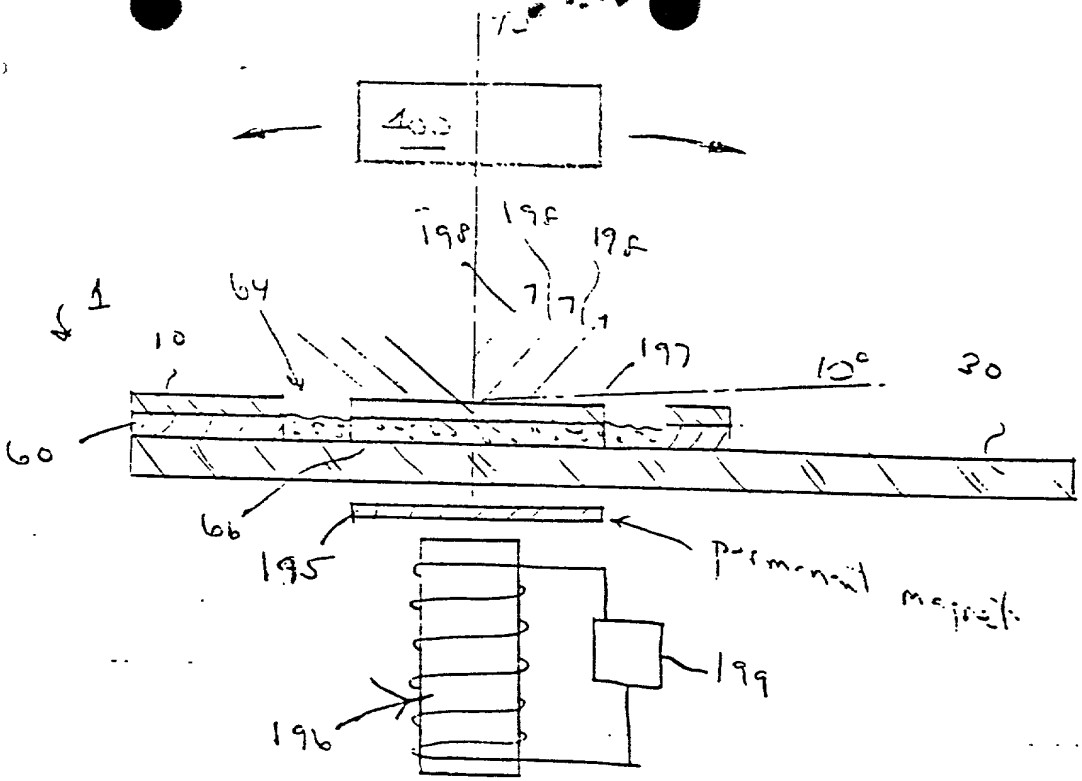


Fig 15